Reduction of Testing on Vertebrates under the Amended Toxic Substances Control Act

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Presentation Outline

- Top 5 study types received by OPPT since 1979
- Amended TSCA 4(h): Alternative Test Methods and Strategies
- EPA New Chemical Categories Document
- Lung Effects Categories
- Examples of Tiered Testing
  - Poly-cationic substances (cationic binding)
  - Surfactants
  - Insoluble polymer lung overload

Expected				Potentially Useful				Available			
Test	**N**	**Test**	**N**	**Test**	**N**	**Test**	**N**	**Test**	**N**	**Test**	**N**
90-D Inhalation	413	Algal Tox	1029	28-day Oral	252	Algal Tox	77	Acute Eye Irri./Corr.	2852	Fish, Acute Tox	4886
Develop. Tox Study	309	Fish Acute Toxicity Test	794	Develop. Tox Study	233	Fish Acute Toxicity Test	61	Acute Dermal Irrit./Corro.	2169	Aquatic Invert. Acute Tox	4287
28-day Oral Tox	243	Aquatic Invert. Acute Tox	773	90-D Inhalation	94	Aquatic Invert. Acute Tox	56	Skin Sensitis	1640	Algal Tox	2990
Cancer Study	226	Fish Early Life Stage Tox	472	Combined Rep Dose/Repro/ Develop Screen	62	Fish Early Life Stage Toxicity Test	43	28-day Oral Tox	1301	Microbial Tox	503
Combined Rep Dose/Repro/ Develop Screen	153	Daphnid Chronic Toxicity Test	467	Mammalian Erythrocyte Micronucleus Test	47	Daphnid Chronic Toxicity Test	36	Acute Dermal Toxicity	1211	14-d Fish Tox	286

Amended TSCA: Section 4(h)

Section 4(h)(1) - “The Administrator shall reduce and replace, to the extent practicable, scientifically justified, and consistent with the policies of this title, the use of vertebrate animals in the testing of chemical substances or mixtures…”

And, for voluntary testing is regulated by 4(h)(3):

Section 4(h)(3)(A) – "Any person developing information for submission under this title on a voluntary basis and not pursuant to any request or requirement by the Administrator shall first attempt to develop the information by means of an alternative test method or strategy identified by the Administrator pursuant to paragraph (2)(C), if the Administrator has identified such a test method or strategy for the development of such information, before conducting new vertebrate animal testing."

EPA New Chemical Categories

EPA's Office of Pollution Prevention and Toxics (OPPT) groups chemicals with shared chemical and toxicological properties into categories to facilitate reviews and determinations on Pre-Manufacture Notices (PMN).

PMN submitters and EPA reviewers benefit from the accumulated data and past decisions represented by a category.

EPA considers all PMNs, including new chemical substances which fall within such categories, on a case-by-case basis and uses the most appropriate structural analogue to support any concerns for health or environmental effects.

Chemical Categories

Category statements were developed for over 50 chemical categories.

- https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf

Lung Effects Categories Project

Short-term reactive process; chemicals disrupt or bind to lung membranes

- Polycationic Substances (Cationic Binding)
- General Surfactants

Longer-term physical process; insoluble polymers may persist in the lungs, leading to lung overload, sustained inflammation, and secondary effects

- Insoluble Polymer Lung Overload

Poly-cationic Substances (Cationic Binding)

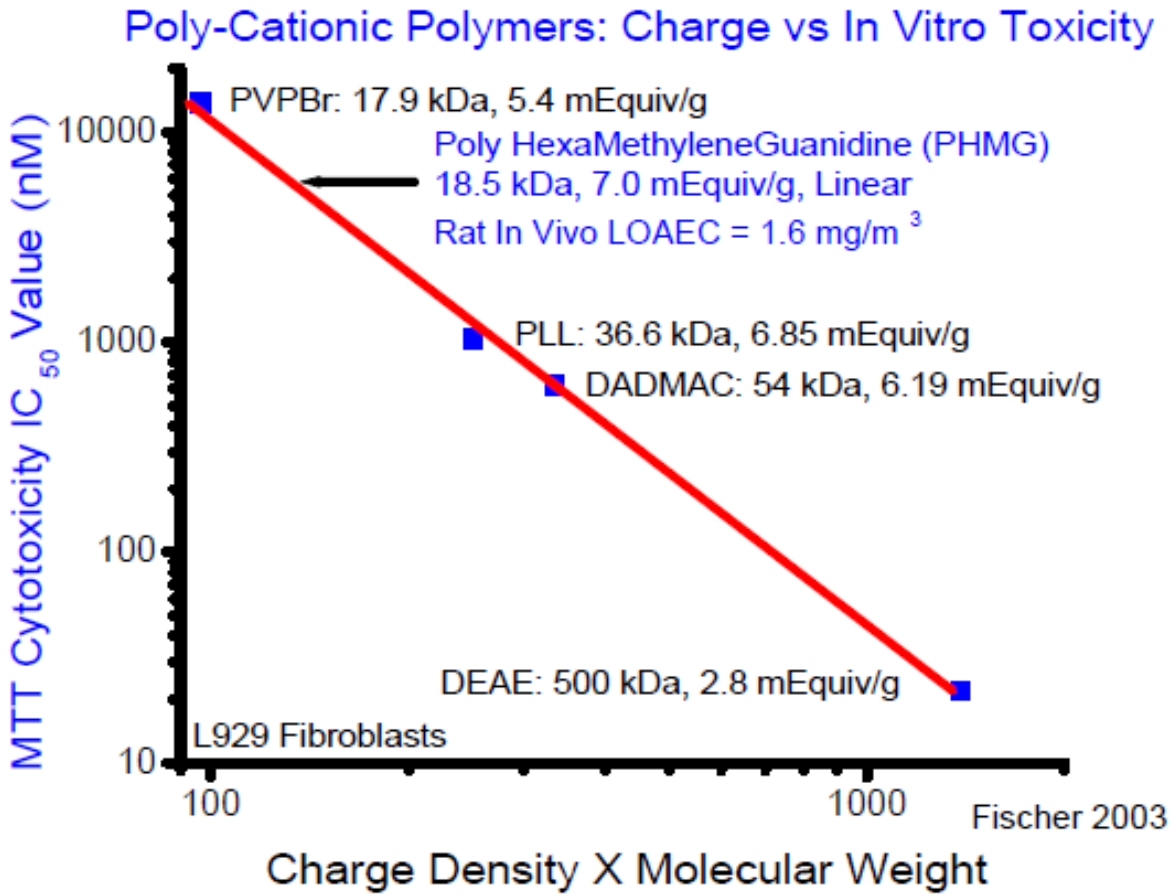
Chemical Space

- Includes: PHMG (polyhexamethylene guanidine), PHMB (polyhexamethylbiguanidine), and quaternary ammonium polymers

Biological Effects and Mode of Action

- Involves electrostatic interaction with pulmonary cell membranes which can result in disruption of lipid bilayers, membrane thinning and nano-scale hole formation (Hong, S., *et al*., 2006)
- Can lead to interstitial lung disease characterized by pneumonia (*i.e.*, swirls of inflammatory tissue filling the alveoli and alveolar ducts) and bronchiolitis obliterans (*i.e.*, swirls or plugs of fibrous granulation tissues filling the bronchioles; Moya *et al*., 1994; Ould Kadi *et al*., 1994)
- Biological effects of category members can be highly variable

Poly-Cationic Substances *In Vitro* Effects



Lower IC_{50} Values
Designates Higher Toxicity

Poly-cationic Substances: Proposed Tiered Testing

Tier I – Use physical-chemical properties to characterize lung exposure/binding potential:

- Charge density in milli-Equivalents/gram or functional group equivalent weight or % amine nitrogen, if estimated IC_{50} is $\geq$ 40X higher than PHMG, stop at Tier I

- Particle Size Distribution or Aerosolized Droplet Size [*i.e.*, cascade impactor, laser methods; OECD Test Guideline (TG) 110, OPPTS 830.7520, OECD Guidance Document (GD) 39]: if no respirable droplets below 10 µm, stop at Tier I

- *If IC_{50} < 40X PHMG and respirable particles or aerosols can be generated during manufacturing, processing, or any of the uses, rodent inhalation toxicity testing may be required under subsequent tiered testing*.

General Surfactants: Anionic, Cationic, & Non-Ionic Types

Chemical Space

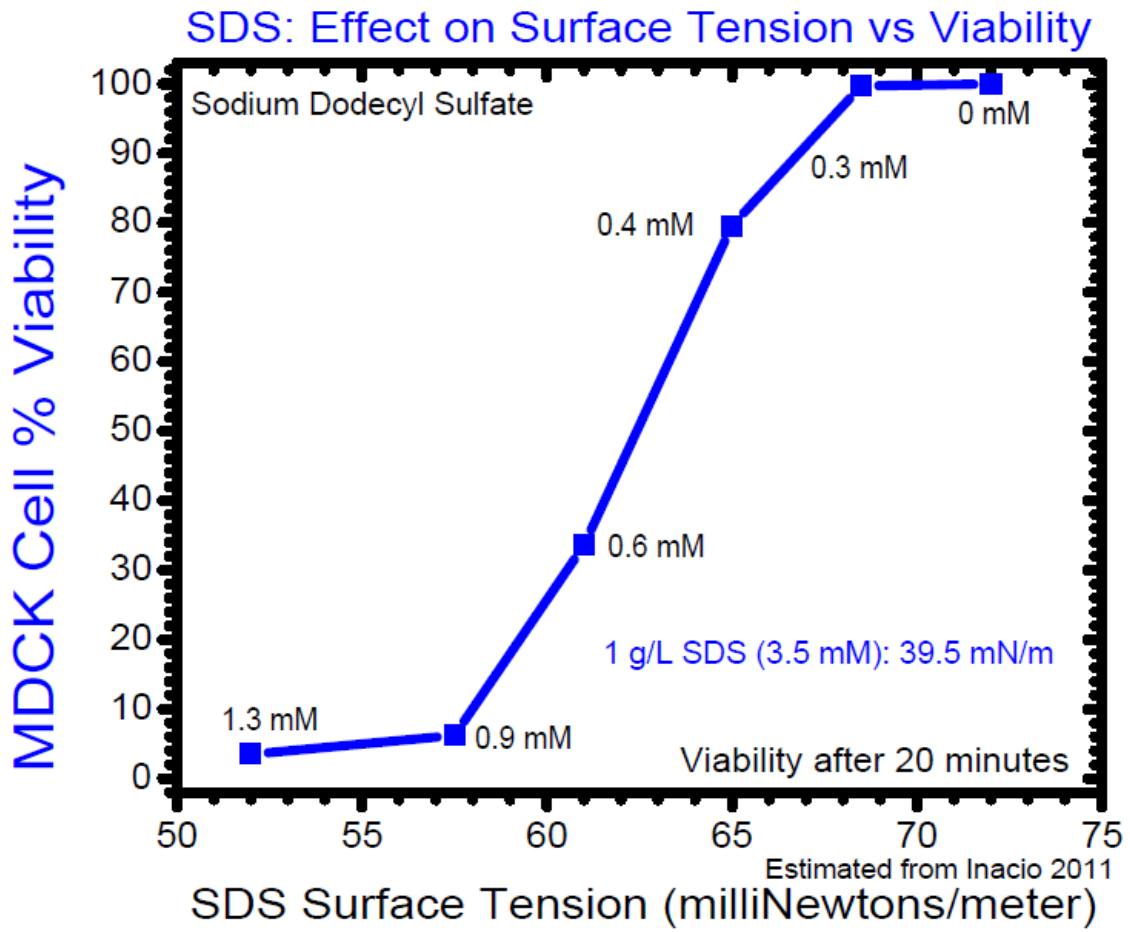
- Includes anionic, cationic, and non-ionic surfactants
- General surfactants are defined by the European Commission as substances that meet all 3 of the following criteria:
  - A substance which has surface active properties and which consists of one or more hydrophilic and one or more hydrophobic groups;
  - The substance must be capable of reducing the surface tension between air and water to 45 mN/m or below at a test condition of 0.5 wt% in water and a temperature of 20 °C; and
  - The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or below
- OPPT is considering adopting these criteria for this category

General Surfactants: Anionic, Cationic, & Non-Ionic Types

Biological Effects and Mode of Action

- Interfere with natural surfactants, resulting in decreased oxygen uptake
- Dysfunction of natural surfactant caused increased alveolar permeability
- Other pulmonary effects included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis (*i.e.*, collapsed alveoli; Nieman and Bredenberg, 1985; Wang *et al*., 1993; Modell *et al*., 1969).
- Can cause cellular membrane disruption at higher exposures

In Vitro Effects vs Dose and Surface Tension



General Surfactants: Proposed Tiered Testing

Tier I – Use physical-chemical properties to characterize lung exposure/disruption

- Surface Tension Measurement (tensiometer using the ring, stirrup or plate method or the capillary surface tension method with appropriate positive controls; ASTM D1331, ASTM D7490, ASTM D3825, OECD TG115). The test concentrations should be 0.1%, 0.5% and 1.0%. Test concentrations representative of those at the unit operation should be tested. In addition, surface tension measurements should be done for the 90% saturated solution concentration for chemical substances with low solubility, if appropriate. If surface tension at 0.5% is reduced to no lower than 45 mN/m, then stop at Tier I.

- Particle Size Distribution or Aerosolized Droplet Size [*i.e.*, cascade impactor, laser methods; OECD TG 110, OPPTS 830.7520, OECD Guidance Document (GD) 39], if no droplets less than 10 µm, then stop at Tier I

- *If respirable particles or aerosols can be generated during manufacturing, processing, or any of the uses and surface tension decreases are observed, rodent inhalation toxicity testing may be required under subsequent tiered testing*.

Insoluble Polymer Lung Overload

Chemical Space

- Includes: insoluble, respirable polymers: polyacrylates, polyvinyls, *etc*.

Biological Effects and Mode of Action

- Insoluble polymers may persist in the lungs: physical, non-reactive process, but may lead to lung overload, irritation, sustained inflammatory response, and secondary effects (Muhle *et al*., 1990a; Bellmann *et al*., 1991)
- Particles less than 10 µm are assumed to enter the deep lungs
- Effects from sustained inflammation due to long-term inhalation exposure to concentrations producing high lung burdens

Polymer Lung Overload: Proposed Tiered Testing

Tier I – Use physical-chemical properties to demonstrate lung exposure:

- Particle Size Distribution or Aerosolized Droplet Size [*i.e.,* cascade impactor, laser methods; OECD TG 110, OPPTS 830.7520, OECD Guidance Document (GD) 39], if no particles less than 10 µm, stop at Tier I
- Bio-solubility Testing* (*i.e.,* solubility or dispersibility in simulated epithelial lung fluid), Employ a simple exponential decay model to predict solubility in Gambles or SELF and polymer half-life: $P(t)=P0e^{-rt}$, where: P(t) = the amount of some quantity at time t; P0 = initial amount at time t = 0; r = the decay rate; t = time. If dissolution rate is higher than the daily exposure estimate, then stop at Tier I
- Determine clearance times and lung burden estimates using *in silico* modeling algorithms (*e.g.*, Multiple-Path Particle Dosimetry (MPPD). If clearance half life is less than 60 days, then stop at Tier I.
- *If respirable and poorly soluble particles can be generated during manufacturing, processing, or any of the uses, rodent inhalation toxicity testing may be required under subsequent tiered testing.*

Questions?